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INHALATION TOXICOLOGY OF RED AND VIOLET MIXTURES
Chamber Concentration and Particle Size Distribution Report

M.A. Higuchi

MANTECH ENVIRONMENTAL TECHNOLOGY, INC.
2 TRIANGLE DRIVE
RESEARCH TRIANGLE PARK, NC 27709

David W. Davies

Principal Investigator: Daniel L. Costa, Sc.D.

PULMONARY TOXICOLOGY BRANCH
ENVIRONMENTAL TOXICOLOGY DIVISION
HEALTH EFFECTS RESEARCH LABORATORY
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

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An inhalation exposure facility was developed at the U.S. Environmental Protection Agency, Research Triangle Park, NC, to conduct inhalation exposures of rodents and guinea pigs to dye mixtures used by the U.S. Army in the manufacture of smoke munitions. Initially, an evaluation of the prototype chamber aerosol homogeneity was conducted to determine the uniformity and reproducibility of the concentration and particle size of dye aerosol throughout the breathing zone of the test animals (Davies, et al.)

The red grenade mixture was formulated by combining the anthraquinone dye Disperse Red 11 (DR11) and the azo dye Solvent Red 1 (SR1); the violet grenade mixture was formulated from DR11 and Disperse Blue 3 (DB3), another anthraquinone dye.

The three dyes, DR11, SR1, and DB3, were chemically analyzed for purity and optically examined for size and shape. All pure dyes appeared to be stable at room temperature except DB3, which decomposes if not stored at 4 °C. The particle size ranges varied for each pure dye and structures were either amorphous (azo dye) or crystalline (anthraquinone dyes).

The bulk red and violet dye mixtures were analyzed for composition. The chemical analysis of the relative composition of each dye mixture, collected by cascade impactor sampling, revealed fractionation of the mixtures into component dyes. The particle size distribution results indicated that the aerosolized RDM had formed a trimodal distribution with MMADs of 0.203, 2.10, and 20.3 µm and with corresponding σ_gs of 1.44, 1.82, and 1.10 µm.

Chamber distribution testing demonstrated that all chambers for both dye mixtures were within or close to acceptable limits for homogeneity of concentrations within the breathing zone of the animal.

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Daniel Costa 3/15/91

Daniel L. Costa, Sc.D. Date
Principal Investigator

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EXECUTIVE SUMMARY

The U.S. Army and the U.S. Environmental Protection Agency (U.S. EPA) entered into an interagency agreement to study the health effects of inhalation exposures to dye mixtures on guinea pigs and rats. The dye mixtures are utilized by the U.S. Army in the manufacture of colored smoke munitions (M18 grenades). The U.S. Army Biomedical Research and Development Laboratory provided funding to the Pulmonary Toxicology Branch of the Environmental Toxicology Division of the U.S. EPA Health Effects Research Laboratory in Research Triangle Park, NC, to conduct toxicological evaluations of laboratory rodents exposed to the dye mixtures by inhalation. This dye aerosol chamber concentration and particle size distribution report describes the results of particle size distributions and chamber aerosol homogeneity tests conducted for the dye mixtures.

Each of the two dye mixtures to be studied is a binary formulation of two dyes. Formulation and mixing of the dyes was performed by the U.S. Army Chemical Research Development and Engineering Center, Munitions Directorate, Production Division, Aberdeen Proving Ground, MD. The red grenade mixture was formulated by combining the anthraquinone dye Disperse Red 11 (DR11) and the azo dye Solvent Red 1 (SR1); the violet grenade mixture was formulated from DR11 and Disperse Blue 3 (DB3), another anthraquinone dye. For field use, additional components are incorporated into the mixture to enhance the burning properties. During the grenade loading, assembly, and packing processes, plant personnel may be incidentally exposed, by inhalation or dermal contact, to the dye mixtures. Toxicology studies with laboratory rodents or guinea pigs are designed to simulate both types of exposures. A previously submitted report (Davies and Higuchi, 1989) describes the inhalation exposure system. This report describes the exposure chamber homogeneity studies.

Initially, an evaluation of the prototype chamber aerosol homogeneity was conducted to determine the uniformity and reproducibility of the concentration and particle size of dye aerosol throughout the breathing zone of the test animals (Davies and Higuchi, 1989). The original number

of 32 rats to be used during the 90-day study with red dye mixture was increased to 96 rats, which is a full chamber load. The aerosol distributions had to be repeated with a full compliment of animals. All of the results from the distribution tests using both dyes are discussed in this report.

The three dyes, DR11, SR1, and DB3, were chemically analyzed for purity and optically examined for size and shape. The bulk red and violet dye mixtures were analyzed for composition. All pure dyes appeared to be stable at room temperature except DB3, which decomposes if not stored at 4 °C. The particle size ranges varied for each pure dye and shapes were of either amorphous (azo dye) or crystalline (anthraquinone dyes) structures.

Chamber distribution testing demonstrated that all chambers (Hazleton 2000) for both dye mixtures were within or close to acceptable limits for homogeneity of concentrations within the breathing zone of the animals. Additionally, animal rotation frequency was increased within the individual housing units of the chamber to accommodate the close-to-acceptable values of the tests.

The chemical analysis of the relative composition of each dye mixture collected by cascade impactor sampling revealed fractionation of the mixtures into component dyes. The ratio of component dyes for each dye mixture changed from that of the bulk dye mixture as particle size decreased. The bulk violet dye mixture had an approximate ratio of 9:1 (DR11:DB3) while the ratio of DR11:DB3 in the aerosol was approximately 5:5 with a mass median aerodynamic diameter (MMAD) of 1.1 µm. The bulk red dye mixture had an approximate ratio of 1:9 (DR11:SR1) and the ratio of DR11:SR1 in the aerosol was approximately 0.05:9.95 with an MMAD of 0.4 µm. Additionally, the relative ratio of component dyes in the aerosol for eacn dye mixture's particle size distribution did not change with chamber concentration.

This analysis led to the hypothesis that the dye mixtures were forming multiple particle size distributions within the chamber. To prove this, particle size distribution data collected by cascade impactor for the red dye mixture was reanalyzed. In addition, several other cascade impactor designs were utilized to confirm this hypothesis. The results indicated that the aerosolized red dye mixture

had formed a trimodal distribution with MMADs of 0.203, 2.10, and 20.3 μm and with corresponding geometric standard deviations of 1.44, 1.82, and 1.10 μm.

SECTION 1

INTRODUCTION

The inhalation exposures to red and violet dye mixtures require that chamber distribution studies be performed to determine aerosol exposure variability. The chamber used for these studies was the Hazleton 2000 (Model 2000, Lab Products, Marywood, NJ), which was designed for aerosol animal exposures (Beethe et al., 1979). The two dye mixtures tested, violet dye mixture (VDM) and red dye mixture (RDM), are mixtures of either anthraquinone or azo dyes. The aerosol was generated using compressed-air grinding mills working in conjunction with dry material feeders that delivered the dye mixtures to the mills. Aerosol distribution studies (30 to 300 mg/m^3) were performed in each of the exposure chambers. These distribution studies were conducted at the study concentrations (30, 100, and 300 mg/m^3) specified for each chamber.

The study design called for measuring the aerosol concentration just above the breathing zone of the cages. All measurements were made by gravimetric analysis using open-face glass fiber filters. In addition, particle size analysis and chemical characterization at specific cut-off points of the distributions were performed at each concentration for both dyes studied.

SECTION 2

STUDY DESIGN

2.1 CHAMBER MEASUREMENTS

2.1.1 Exposure Chamber

The Hazleton 2000 inhalation chamber was used to conduct all the distribution studies for both dye mixtures. The configuration of the chamber (Figure 1) was arranged with three tiers of cage modules and two modules on each tier. There were six sample locations on the front and six on the back of the chamber, with each position located immediately above the breathing zone of the animal cage.

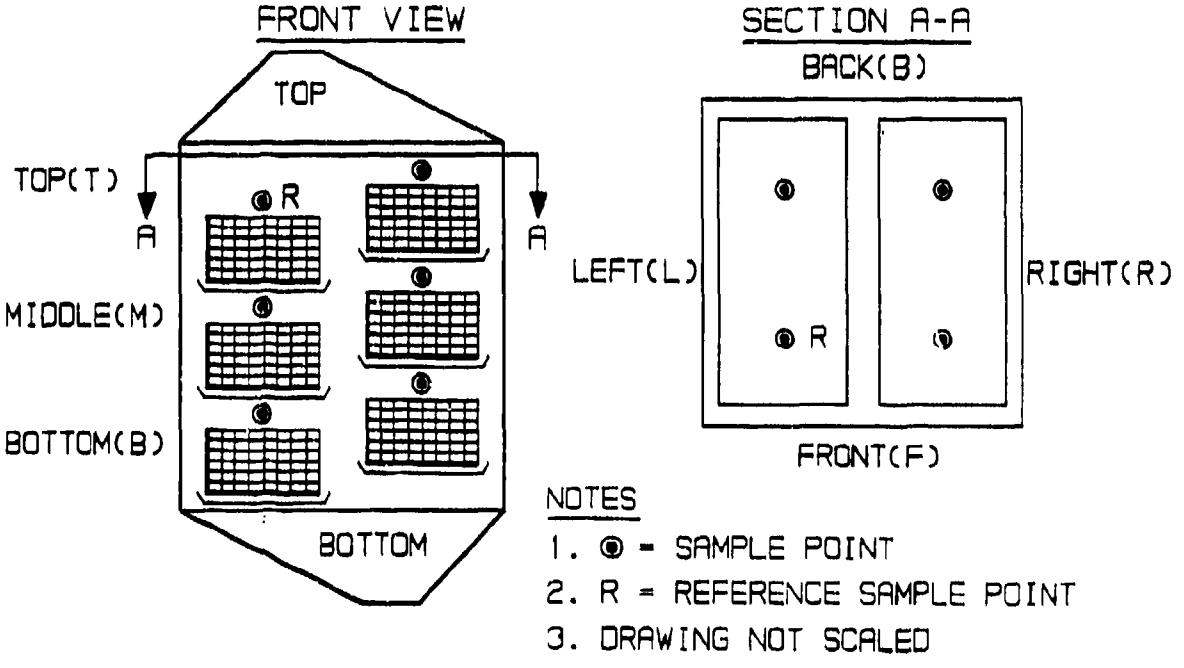


Figure 1. Sample Probe Locations for Chamber Distribution Test.

The dilution air flow plus the flow from the aerosol generator was maintained at a constant rate of 500 ℓ/min through the exposure chamber. This was monitored by a calibrated orifice plate located in the chamber exhaust line. The dry air from the generator was mixed with filtered, humidified (40 to 70% relative humidity), and temperature-conditioned (72 ± 2 °F) air before entering the chamber (Davies and Higuchi, 1989).

2.1.2 Sample Trains

All sampling was conducted by gravimetric analysis using 25-mm open-face Delrin filter holders (Gelman Sciences, Ann Arbor, MI) and type-A/E, glass-fiber filters (Gelman Sciences, Ann Arbor, MI). All 12 chamber locations were sampled simultaneously using two sampling manifolds with each manifold containing six individually calibrated critical orifices (~1.0 ℓ/min). The sample lines from each critical orifice were the same length to ensure equal flow through all open-face filters. Each vacuum manifold was connected to a vacuum solenoid controlled by a timer (Figure 2).

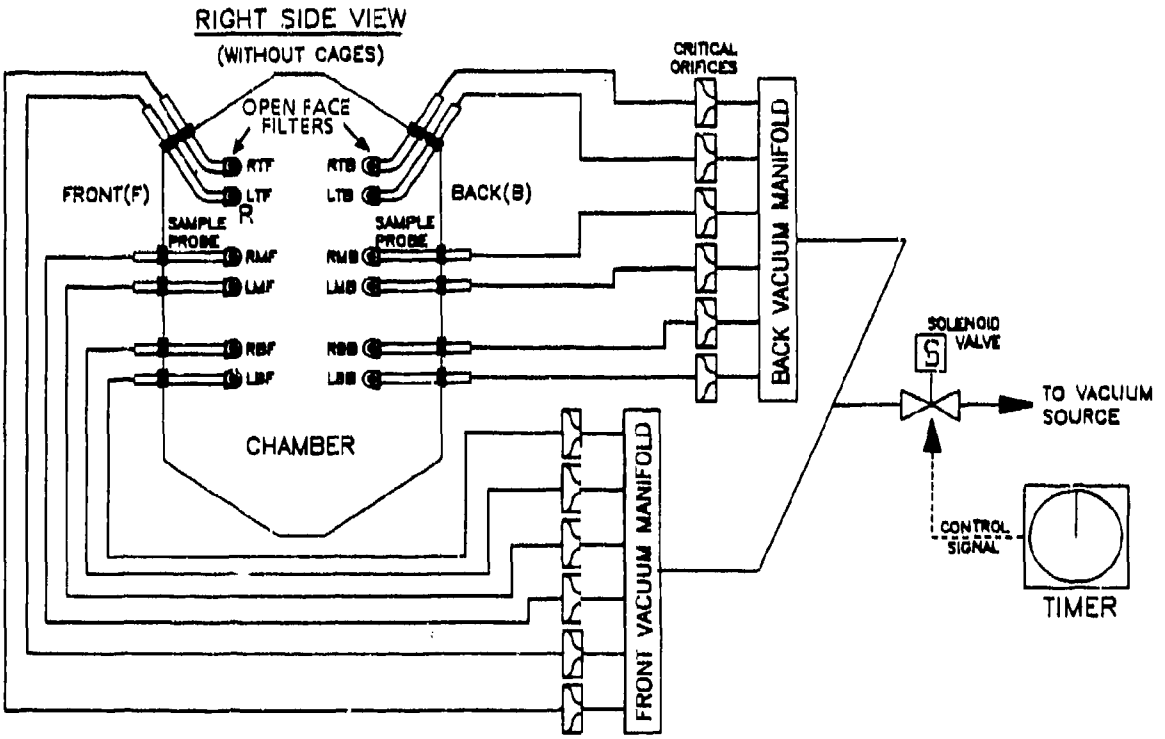


Figure 2. Distribution Sampling System.

The sample time was chosen based on the chamber aerosol concentration. Optimum total aerosol mass collected on the filters was determined based on filter loading and gravimetric balance limits. This allowed for accurate quantification at all concentrations using a semimicro balance (Model AE 240, Mettler Instrument Corp., Hightstown, NJ). Then the sample time was adjusted to collect the optimum mass at each aerosol concentration.

The two sample manifolds supplied 12 mass concentration measurements averaged over various time spans. These include 15 min at 300 mg/m^3; 30 min at 100 mg/m^3; and 45 min at 70, 40, and 30 mg/m^3 using a sample rate of approximately 1.0 ℓ/min. These 12 measurements were repeated three times at each concentration level to give a total of 36 readings. Sample position "R" (Figure 1) was the reference position for all concentration levels. The sampling system and the location of all sample probes is shown in Figure 2.

2.1.3 Particle Size Distributions

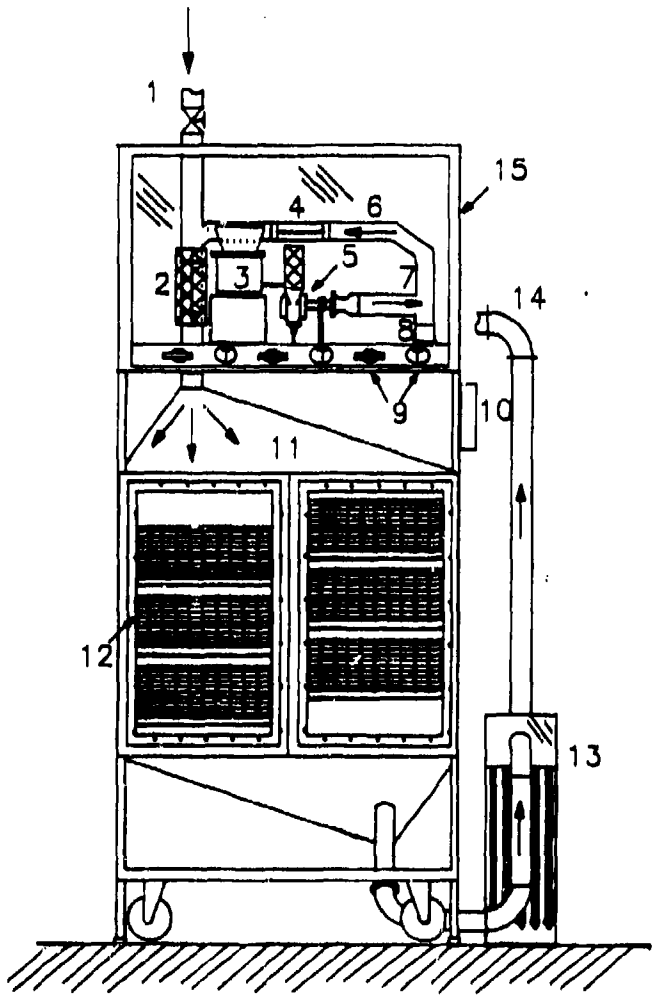
Each pure dye was examined by scanning electron microscopy (SEM) to determine the average (bulk powder) starting particle size and shape. The dyes, which were previously mixed by the U.S. Army, were ground by jet mill, delivered to the chamber, and analyzed for particle size and chemical composition.

During each chamber aerosol homogeneity test, particle size distributions were performed to identify fractional components of the dye mixtures. The instrument used for this measurement was a cascade impactor (1 actual ft^3/min Ambient Impactor [Nonviable], Andersen, Atlanta, GA) connected to a dry gas meter with a critical orifice (1.0 ft^3/min) installed. This system was turned on and off by a vacuum solenoid connected to a timer to vary the amount of sampling time for each concentration level. Additionally, a low pressure cascade impactor (Andersen, Atlanta, GA) and a seven-stage cascade impactor (Inhalation Toxicology Products [ITP], Albuquerque, NM) were used to compare different size cut points.

2.2 GENERATION SYSTEM

The generation system diagrams shown in Figure 3 (front view) and Figure 4 (side view) include all the major components. Either dye mixture was delivered to the jet mill (Jet-O-Mizer Model 0101, Fluid Energy Processing & Equipment, Hatfield, PA) by a modified dry material feeder (Model 106, AccuRate Inc., Whitewater, WI) to improve the relative standard deviations of feed rates (Higuchi and Steinhagen, 1991). The modification also prevented the feeder helix from slowing in revolutions per

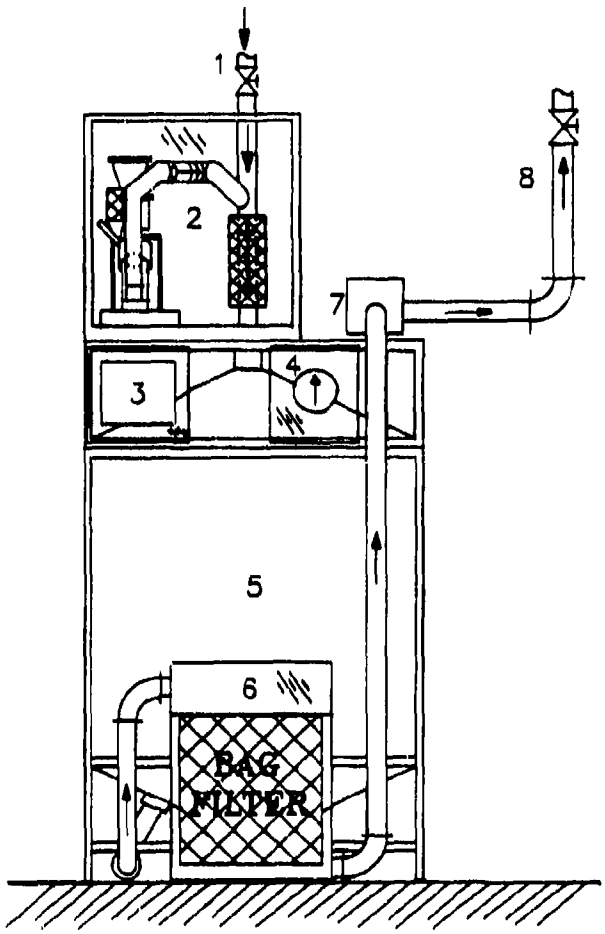
minute or completely binding in the nozzle. The jet mill ground the dye and sent material to a single-stage impactor for further classification of particle sizes. The dye aerosol passed through one of two Kr^{85} neutralizers to reduce particle charging before being mixed with humidified dilution air. The dye aerosol passed through a combined silencer/Kr^{85} particle neutralizer to reduce jet mill noise and any possible static charge before diffusing around a baffle plate mounted at the chamber entrance nozzle. The generation system was operated at approximately 300 ℓ/min and under a slight negative pressure (0.1 in. of water) to ensure aerosol containment within the generation lines and the exposure chamber.



LEGEND

1) CHAMBER DILUTION AIR
2) GENERATOR SILENCER WITH RADIOACTIVE ELEMENT
3) DRY POWDER FEEDER
4) AEROSOL OBSERVATION WINDOW WITH RADIOACTIVE ELEMENT
5) JET MILL
6) AEROSOL INJECTION LINE
7) IMPACTOR
8) LARGE PARTICLE COLLECTION CUP
9) JET MILL AIR PRESSURE CONTROL VALVES AND GAUGES
10) DRY POWDER FEEDER CONTROLLER
11) H-2000 EXPOSURE CHAMBER
12) ANIMAL EXPOSURE CAGE
13) BAG FILTER AND HOUSING
14) EXHAUST LINE
15) GENERATOR CONTAINMENT

Figure 3. Exposure System – Front View.



LEGEND

1) CHAMBER DILUTION AIR
2) AEROSOL GENERATOR
3) DRY POWDER FEEDER CONTROLLER
4) MAGNEHELIC VACUUM GAUGE
5) ANIMAL EXPOSURE CHAMBER
6) BAG FILTER AND HOUSING
7) HEPA FILTER
8) EXHAUST LINE

Figure 4. Exposure System – Side View.

2.3 EXPERIMENTAL DESIGN

The RDM chamber aerosol distributions were examined at concentrations of 300, 100, and 30 mg/m^3 with and without animals. The VDM aerosol distributions were conducted at 300, 100, 70, and 40 mg/m^3 without animals in the chamber.

The statistical design of the chamber distribution experiments examined four parameters: total port variability (TPV), within port variability (WPV), between port variability (BPV), and sampling instrument variability (SIV). The TPV is the total measured variability between sampling ports. The WPV represents the fluctuation of the average concentration during the time of chamber balance measurements. The BPV is the actual variability between sampling ports, which is calculated from the

TPV and the WPV. The SIV was always shown to be small (<0.5%). The normally acceptable limits for each of these parameters when generating a gas or vapor are TPV ≤ 7%, WPV ≤ 5%, and BPV ≤ 5%, but due to the difficulty in generating large aerosols in large exposure chambers at high concentrations, the acceptable limits for aerosols are usually twice as great, or TPV ≤ 14%, WPV ≤ 10%, and BPV ≤ 10% (McClellan and Henderson, 1989).

Particle size distributions were conducted for all concentration levels of RDM and at 70 and 300 mg/m^3 for VDM. Additional particle size distributions using two other cascade impactor designs (low pressure and ITP seven-stage) were conducted at 300 mg/m^3 using RDM with animals. Chemical analysis of the dye sample on each stage of the cascade impactor was performed at 300 mg/m^3 using RDM (with animals present) to identify the percentage of each dye component at the effective cut point for that stage.

SECTION 3

RESULTS AND DISCUSSION

3.1 VDM CHAMBER CONCENTRATION DISTRIBUTION

The VDM was tested at four concentration levels with no animals in the chamber and the results are shown in Table 1. These studies were not repeated with animals in the chamber because the toxicity of the VDM by whole-body exposure was found to be too high. All distribution studies for each aerosol concentration level were conducted in the same chamber.

TABLE 1. VDM CHAMBER AND PARTICLE SIZE DISTRIBUTION RESULTS[a]

Concentration (mg/m^3)	WPV (%)	TPV (%)	BPV (%)	MMAD (μm)	σ_g (μm)
40	11.1	24.6	22.0		
70	10.6	25.2	22.8	3.60	2.06
100	5.8	26.0	25.3		
300	3.0	22.6	22.4	5.60	2.14

[a] All VDM distributions and animal exposures were conducted in Chamber #4. All WPVs for VDM distributions were from a common reference position in geometric center of chamber.

The TPV was approximately 25% for all concentrations tested, which is two times greater than specified limits for aerosols in these chambers. This high variability may be attributed to the large particle sizes, on the order of 4 μm. Because acute studies with VDM did not involve more than 32 animals, one tier of the chamber provided adequate housing. The variability seen at any location was decreased by maintaining all of the animals on Tier 1 (top level) of the chamber. The results of these single-tier experiments are seen in Table 2.

The WPV remained the same for each chamber distribution, because the average concentration seen at the reference position during chamber balance measurements did not change. The mean TPV for all concentrations at each tier was 9.1, 30.2 and 11.4% for Tiers 1, 2 (middle level), and 3 (bottom level), respectively. This represents a 2.5-fold decrease in Tier 1 total variability when

compared to the average TPV for all concentrations on all chamber tiers. The mean BPV for all concentrations at each tier was 4.7, 29.0, and 7.4% for Tiers 1, 2, and 3, respectively. This indicates almost a fivefold decrease from the average BPV for all concentrations on all chamber tiers when compared to Tier 1 BPV.

TABLE 2. VDM CHAMBER DISTRIBUTION RESULTS AT SINGLE LEVEL[a]

Tier Number	Concentration (mg/m^3)	WPV (%)	TPV (%)	BPV (%)
1	100	5.76	10.42	8.68
2	100	5.76	28.04	27.45
3	100	5.76	20.37	19.54
1	300	2.98	4.65	3.58
2	300	2.98	29.30	29.15
3	300	2.98	3.05	0.65
1	70	10.62	8.49	0.00
2	70	10.62	32.74	30.97
3	70	10.62	14.30	9.57
1	40	11.12	12.96	6.65
2	40	11.12	30.50	28.40
3	40	11.12	8.00	0.00

[a] The chamber distribution sampling on each tier (1, 2, and 3) was conducted simultaneously. The analysis indicates tier differences using a single time reference. All VDM distributions and animal exposures were conducted in Chamber #4.

This indicates that all variability measurements (TPV, WPV, and BPV) are within acceptable limits for Tier 1 at all concentration levels. Only the WPVs at concentrations of 70 and of 40 mg/m^3, measured at 10.6 and 11.1%, respectively, were marginal.

3.2 RDM CHAMBER CONCENTRATION DISTRIBUTION

The RDM was tested at three concentration levels with no animals in the chamber (Table 3) and with animals in the chamber (Table 4). Since the RDM was used to conduct a 13-week subchronic study, the entire chamber was needed to accommodate the additional animal loading. The study required that 96 Fisher-344 rats be exposed at each concentration. The distribution studies used

48 rats spaced evenly thoughout the chamber with the same rats being moved from chamber to chamber. The distribution studies were conducted in different chambers for each concentration level.

TABLE 3. RDM CHAMBER AND PARTICLE SIZE DISTRIBUTION RESULTS WITHOUT ANIMALS[a]

Chamber Number	Concentration (mg/m^3)	WPV (%)	TPV (%)	BPV (%)	MMAD (µm)	σ_g (µm)
2	30	12.1	14.6	8.1	2.15	2.47
3	100	11.6	15.0	9.5	2.25	2.22
4	300	4.9	14.5	13.6	1.90	2.51

[a] Each distribution was conducted in a specific chamber and concentration level. The particle size distributions were performed with an eight-stage cascade impactor (1 actual ft^3/min, Andersen).

TABLE 4. RDM CHAMBER AND PARTICLE SIZE DISTRIBUTION RESULTS WITH ANIMALS[a]

Chamber Number	Concentration (mg/m^3)	WPV (%)	TPV (%)	BPV (%)	MMAD (µm)	σ_g[b] (µm)
2	30	12.6	10.2	9.8	2.30	2.17
3	100	10.7	11.8	5.2	2.25	2.13
4	300	3.8	8.8	7.9	2.25	2.17

[a] Each distribution was conducted in a specific chamber and concentration level. The particle size distributions were performed with an eight-stage cascade impactor (1 actual ft^3/min, Andersen).

[b] Due to the large σ_gs from the 1 actual ft^3/min Andersen impactor, the particle size distributions were repeated with animals using a low pressure impactor (Andersen) and a seven-stage impactor (ITP) to determine the cause for the high σ_gs seen in both distributions.

The distribution results for the empty chamber indicated that almost all variability measurements were marginally unacceptable. When the experiments were repeated with animals (48 rats) all the variabilities were within limits, except for a WPV of 10.7% at 100 mg/m^3. To correct for this insufficiency, the animals were rotated every week instead of every other week.

3.3 PARTICLE SIZE DISTRIBUTIONS

3.3.1 VDM Distribution

The SEM of Disperse Blue (DB3) indicates initial particle sizes ranging from 1.5 to 30 µm (Figure 5) and a crystalline structure. This indicates a broad range of particle sizes and that this dye should be present at almost all effective cut diameters (ECDs). The SEM of Disperse Red 11 (DR11) indicates particle sizes in the range of 0.75 to 40 µm (Figure 6) and is crystalline in structure. Both dyes

have wide particle size ranges, but DR11 has some particles in smaller sizes (0.75 to 1.5 μm). These are the anthraquinone dyes, with corresponding crystalline structures.

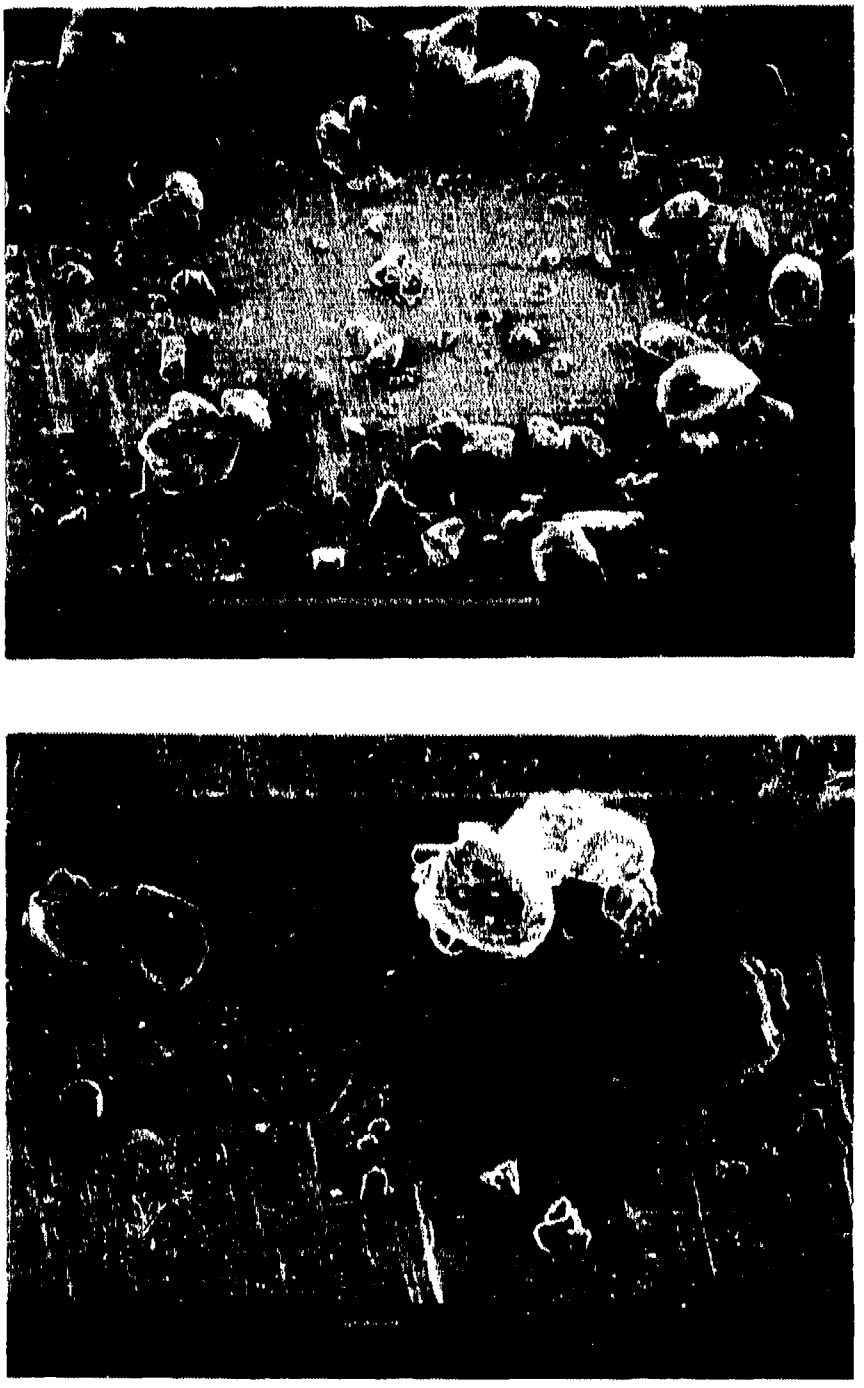


Figure 5. Scanning Electron Microscopy (SEM) of DB3.

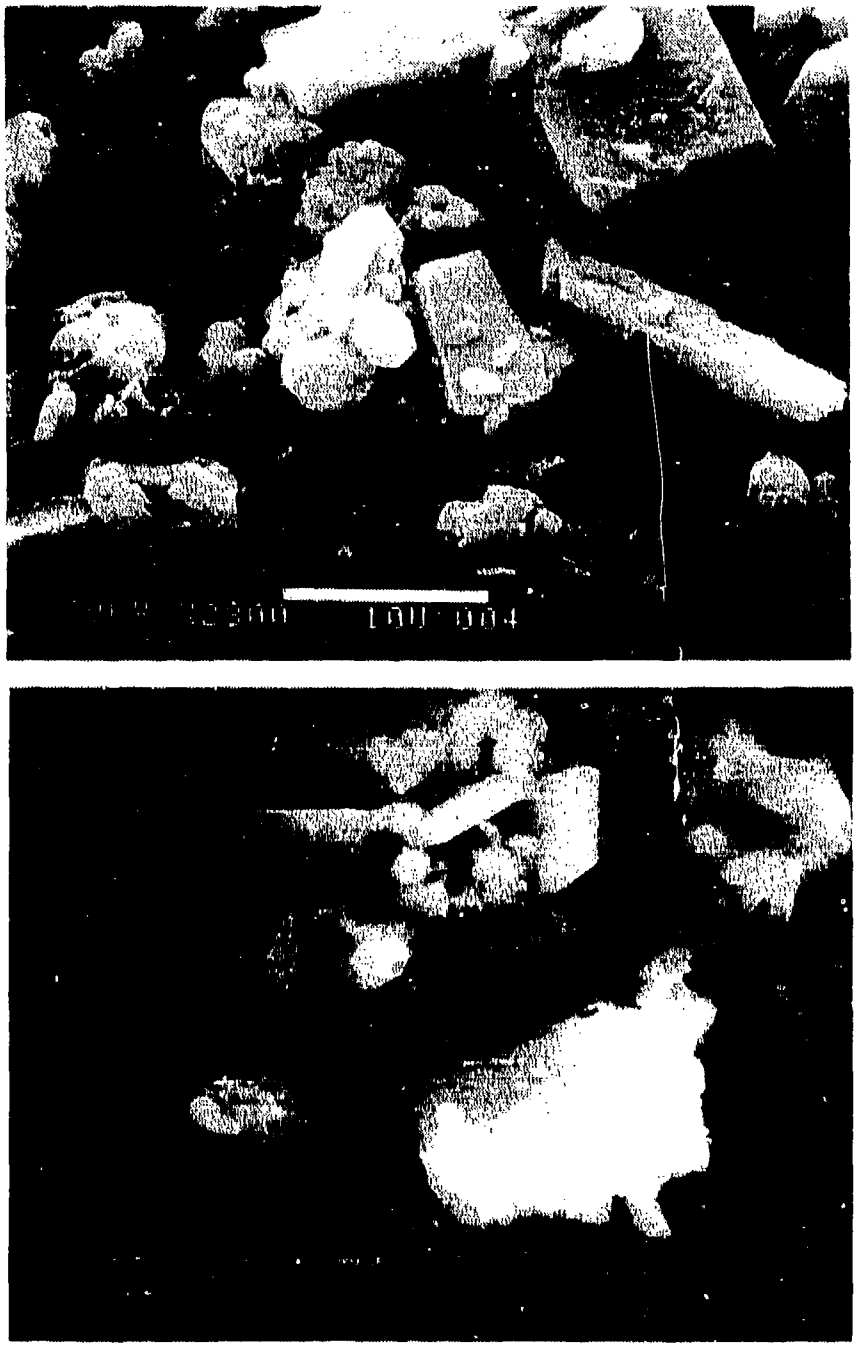


Figure 6. Scanning Electron Microscopy (SEM) of DR11.

The cascade impactor results indicated mass median aerodynamic diameters (MMADs) of 5.6 and 3.6 μm with corresponding geometric standard deviations (σ_gs) of 2.14 and 2.06 μm at 300 and 70 mg/m^3, respectively (Table 1). These large MMADs may be the reason for the large TPVs seen for all concentrations, but further investigation would be required to confirm this hypothesis.

The analysis of the relative composition of VDM on each cascade impactor stage indicated significant differences from the bulk material (Table 5). The two components of the VDM bulk material showed approximately 93% DR11 and 7% DB3 when stored at room temperature (~72 °F).[1] This ratio changed when the dye mixture was aerosolized by the jet mill and particle sized by the cascade impactor. As the particle size decreased, the percentage of DR11 decreased and the percentage of DB3 increased. At an ECD of 1.1 µm (the smallest particle size with detectable amounts), the approximate amounts were 57% DR11 and 43% DB3.

TABLE 5. RELATIVE COMPOSITION OF VDM ON EACH STAGE OF CASCADE IMPACTOR[a]

Sample	Size Range (µm)	ECD[b] (µm)	DR11 (%)	DB3 (%)
VDM Bulk[c]			92.9	7.14
Stage 0 filter	9.0 - 10.0	9.0	88.1	11.9
Stage 1 filter	5.8 - 9.0	5.8	83.4	16.6
Stage 2 filter	4.7 - 5.8	4.7	76.9	23.1
Stage 3 filter	3.3 - 4.7	3.3	63.6	36.4
Stage 4 filter	2.1 - 3.3	2.1	56.0	44.0
Stage 5 filter	1.1 - 2.1	1.1	57.4	42.6
Stage 6 filter	0.7 - 1.1	0.7	d	d
Stage 7 filter	0.4 - 0.7	0.4	d	d
Stage 8 filter	0.0 - 0.4	0.0	d	d

[a] All particle size distributions were performed with an eight-stage cascade impactor (1 actual ft^3/min Andersen). The dye on each stage was extracted with acetonitrile and analyzed by high performance liquid chromatography.

[b] Effective cut diameter

[c] Stored at ambient temperature (72 ± 2 °F)

[d] Below detection limit

3.3.2 RDM Distribution

The SEM of Solvent Red 1 (SR1) indicates particle sizes ranging from 0.25 to 43 µm (Figure 7). These particles are spread over a larger range of sizes and can be considerably smaller than particles of the other dyes. This dye also displays an amorphic structure as opposed to the crystalline structures

[1] The component dye DB3 of the VDM degrades if not stored at 4 °C.

of the anthraquinone dyes. This azo dye has particles with surfaces that contain aggregated plates that shear off during grinding, allowing the formation of very small particles.

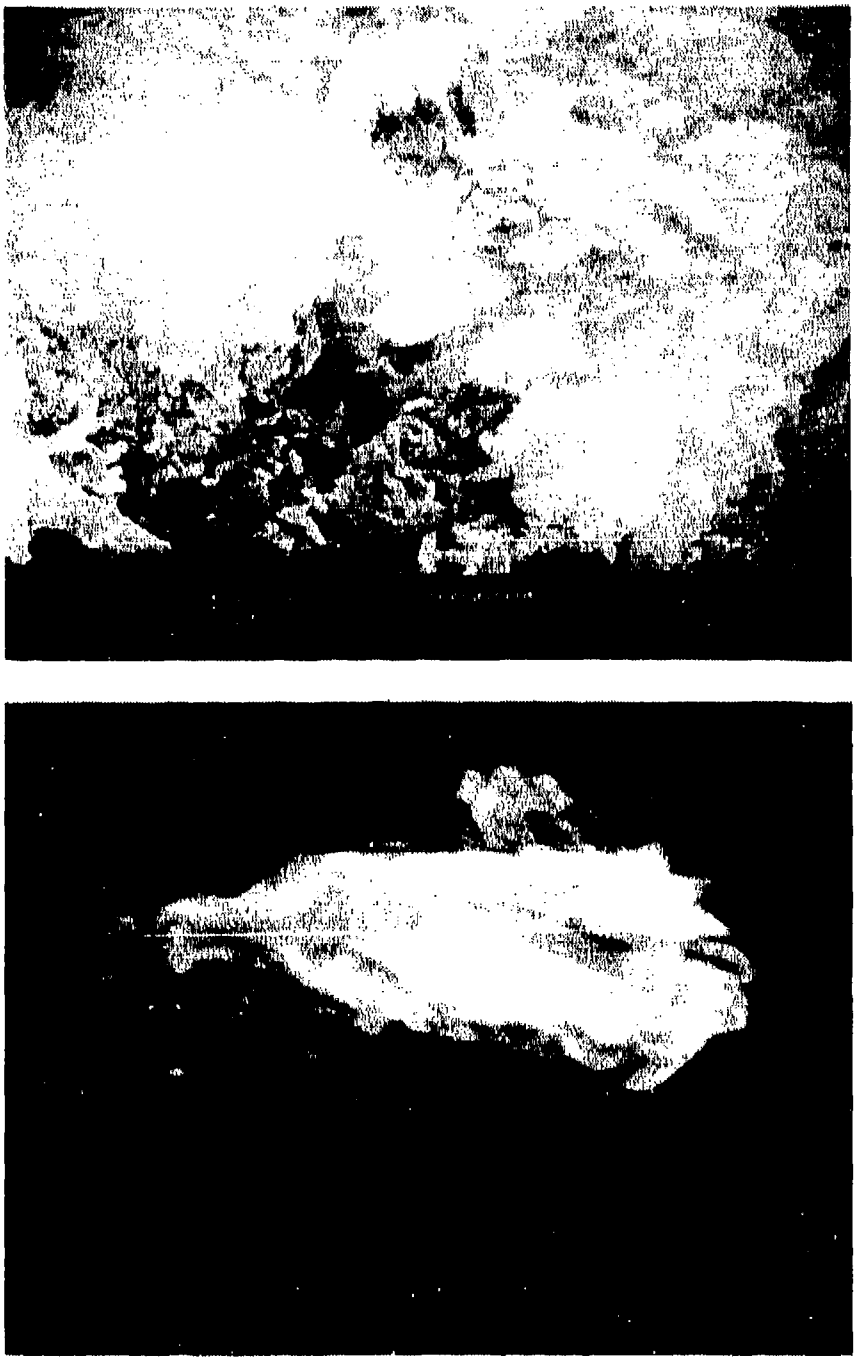


Figure 7. Scanning Electron Microscopy (SEM) of SR1.

The cascade impactor (1 actual ft^3/min, Andersen) results for chambers with no animals present indicated MMADs of 1.9, 2.2, and 2.2 µm with corresponding σ_gs of 2.5, 2.2, and 2.5 µm at concentrations of 300, 100, and 30 mg/m^3, respectively (Table 3). The results for chambers with animals present showed MMADs of 2.2, 2.2, and 2.3 µm with corresponding σ_gs of 2.2, 2.1, and 2.2 µm at concentrations of 300, 100, and 30 mg/m^3, respectively (Table 4). The MMADs did not change with the presence of animals, but the σ_gs did decrease slightly at corresponding concentrations.

The analysis of relative composition of RDM on each cascade impactor stage also indicated significant differences from the bulk material (Table 6). The two components of the RDM bulk material showed approximately 91% SR1 and 9% DR11 when stored at room temperature (~72 °F). This ratio changed when the dye mixture was aerosolized by the jet mill and particle sized by the cascade impactor. As the particle size decreased, the percentage of DR11 decreased and the percentage of SR1 increased. At an ECD of 0.4 µm, the approximate amount of DR11 was 0.4% and SR1 was 99.6%.

TABLE 6. RELATIVE COMPOSITION OF RDM ON EACH STAGE OF CASCADE IMPACTOR[a]

Sample	Size Range (µm)	ECD (µm)	DR11 (%)	SR1 (%)
RDM Bulk A[b]			9.91	90.1
RDM Bulk B[b]			8.91	91.1
Stage 0 filter	9.0 - 10.0	9.0	7.17	92.8
Stage 1 filter	5.8 - 9.0	5.8	6.03	94.0
Stage 2 filter	4.7 - 5.8	4.7	4.80	95.2
Stage 3 filter	3.3 - 4.7	3.3	4.19	95.8
Stage 4 filter	2.1 - 3.3	2.1	3.16	96.8
Stage 5 filter	1.1 - 2.1	1.1	1.76	98.2
Stage 6 filter	0.7 - 1.1	0.7	0.80	99.2
Stage 7 filter	0.4 - 0.7	0.4	0.36	99.6
Stage 8 filter	0.0 - 0.4	0.0	0.45	99.6

[a] All particle size distributions were performed with an eight-stage cascade impactor (1 actual ft^3/min Andersen). The dye on each stage was extracted with acetonitrile and analyzed by high performance liquid chromatography.

[b] Bulk mixtures A and B are the same lot of dye analyzed at different times when stored at room temperature and at 4 °C.

To further determine the reason for high σ_gs seen with the RDM, two other impactors were used: a low pressure impactor and a seven-stage impactor with controlled cut points. Initially, the low pressure impactor was used to determine if bimodal or trimodal distributions were occurring. This was possible and suspected because of the large particle size range (0.07 to 35.0 µm) of the impactor. When the data was analyzed using a log-normal distribution (Distfit, TSI Inc., St. Paul, MN), a trimodal distribution was indicated. The three particle size distributions had MMADs of 0.212, 1.72, and 16.3 µm with corresponding σ_gs of 1.56, 2.05, and 1.64 µm at 300 mg/m^3 (Figure 8 and Table 7).

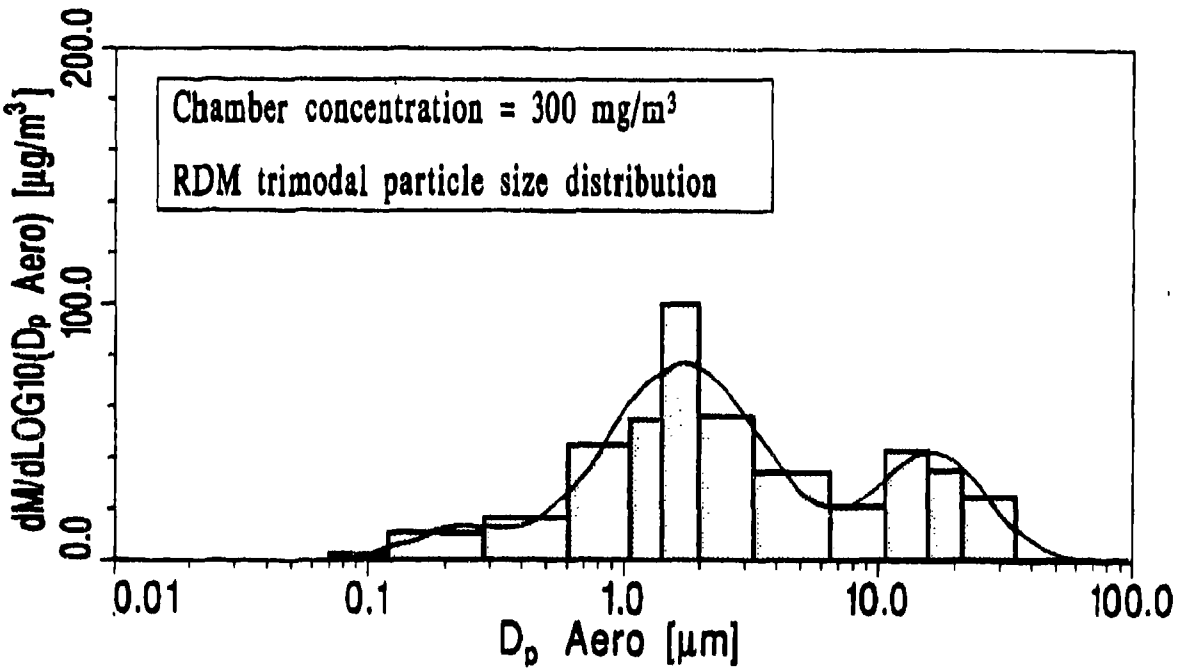


Figure 8. Low Pressure Cascade Impactor Curve.

TABLE 7. DISTFIT REPORT FOR LOW PRESSURE CASCADE IMPACTOR CURVE

Moment type (x-axis): D_p Aero (µm)
Included intervals: 0.070 – 35.0 (µm) (1 – 12)
Weighting type (y-axis): M (µg/m³)
Converted data type: Interval

Geometric Measures of Central Tendency

	Total	Mean	Median	Mode	Std Dev
Discrete data	497.0	7.35	10.1	18.8	3.22
Analytical data	87.1	2.55	2.18	–	3.72
Function	88.5	2.67	2.23	–	3.86

Analytical Fitting Functions

Type	M	MMD	SD_g	ChiSq
1: LNDF	5.94	0.212	1.56	0.715
2: LNDF	59.9	1.72	2.05	0.715
3: LNDF	22.7	16.3	1.64	0.715

Fractional Classifications

Respirable M Fraction	Discrete Data	Analytical Data	Function
ACGIH based on LNDF:	0.266	0.623	0.613
ACGIH based on polynomial:	0.254	0.579	0.569

Size Classification	Discrete Data	Analytical Data	Function
Fraction < 1 (µm):	0.0587	0.219	0.219
Fraction > 1 (µm):	0.949	0.781	0.781

Discrete Data

D_p Aero	Converted Data	Analytical Data	Percent Difference	Correction
0.070	2.81	0.5598	80.08	1.0
0.12	3.37	4.112	-22.03	1.0
0.28	7.49	5.639	24.72	1.0
0.61	13.11	10.29	21.49	1.0
1.05	23.78	8.432	64.54	1.0
1.4	30.71	11.71	61.87	1.0
2.0	46.25	14.07	69.58	1.0
3.3	58.61	9.875	83.15	1.0
6.6	69.28	4.967	92.83	1.0
10.5	73.77	6.702	90.92	1.0
15.7	81.45	5.696	93.01	1.0
21.7	86.51	5.032	94.18	1.0

The marginally high σ_g (>2.0 µm) of the middle mode was believed to be caused by the close proximity of the two other size distributions. Therefore, an ITP seven-stage impactor was used to control for the higher and lower distribution influences. The impactor was run at two different cut point ranges, 7.30 to 0.45 µm and 8.00 to 0.49 µm. These analyses indicated that the MMAD was 2.68 µm and σ_g was 1.82 µm for the first size range, 7.30 to 0.45 µm (Figure 9 and Table 8), and that the MMAD was 1.89 µm and σ_g was 1.83 µm for the second range, 8.00 to 0.49 µm (Figure 10 and Table 9).

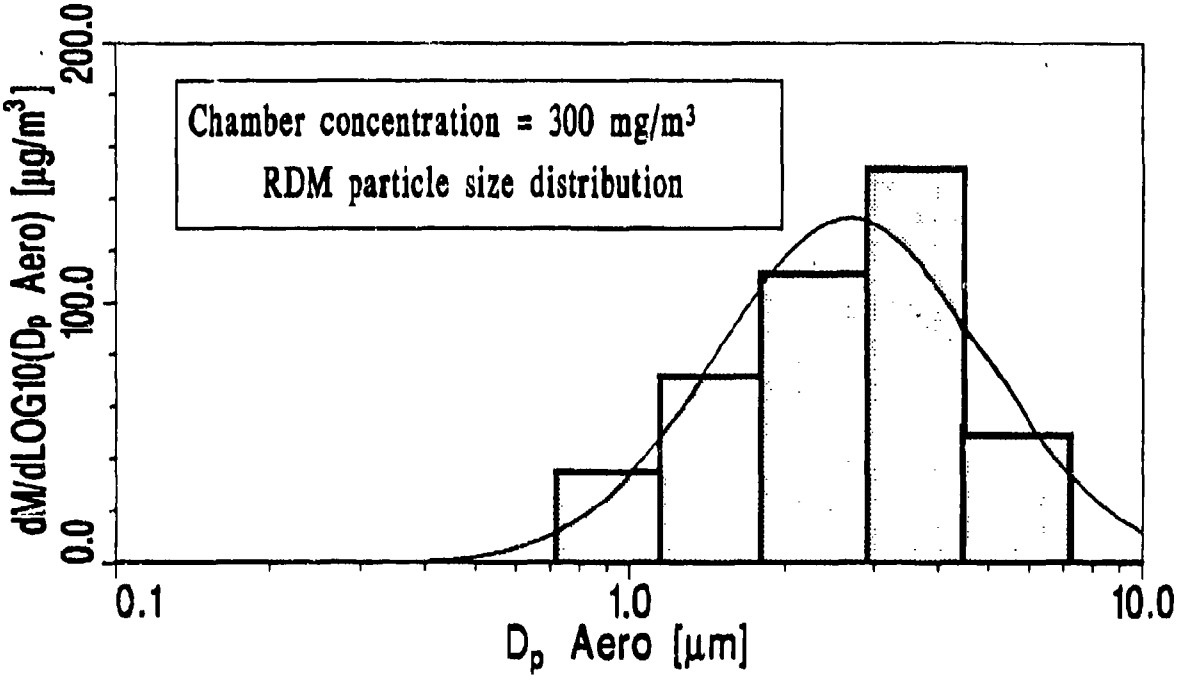


Figure 9. ITP Cascade Impactor Curve (0.45-7.3 µm).

TABLE 8. DISTFIT REPORT FOR ITP CASCADE IMPACTOR CURVE (0.45 to 7.3 μm)

Moment type (x-axis):	D_p Aero (μm)
Included intervals:	0.72 – 7.3 (μm) (1 – 5)
Weighting type (y-axis):	M (μg/m³)
Converted data type:	Interval

Geometric Measures of Central Tendency

	Total	Mean	Median	Mode	Std Dev
Discrete data	163.0	3.88	4.45	0.51	1.6
Analytical data	12.0	1.03	1.11	-	1.3
Function	85.3	2.62	2.68	-	1.78

Analytical Fitting Functions

Type	M	MMD	SD_g	ChiSq
1: LNDF	86.5	2.68	1.82	0.444

Fractional Classifications

Respirable M Fraction	Discrete Data	Analytical Data	Function
ACGIH based on LNDF:	0.4	0.997	0.653
ACGIH based on polynomial:	0.411	0.9	0.627

Size Classification	Discrete Data	Analytical Data	Function
Fraction < 1 (μm):	0.01345	0.04987	0.04987
Fraction > 1 (μm):	0.987	0.95	0.95

Discrete Data

D_p Aero	Converted Data	Analytical Data	Percent Difference	Correction
0.72	3.29	5.42	-64.74	1.0
1.14	10.26	15.78	-53.77	1.0
1.82	24.8	25.18	-1.537	1.0
2.89	47.11	22.96	51.26	1.0
4.59	77.59	11.88	84.69	1.0
7.3				

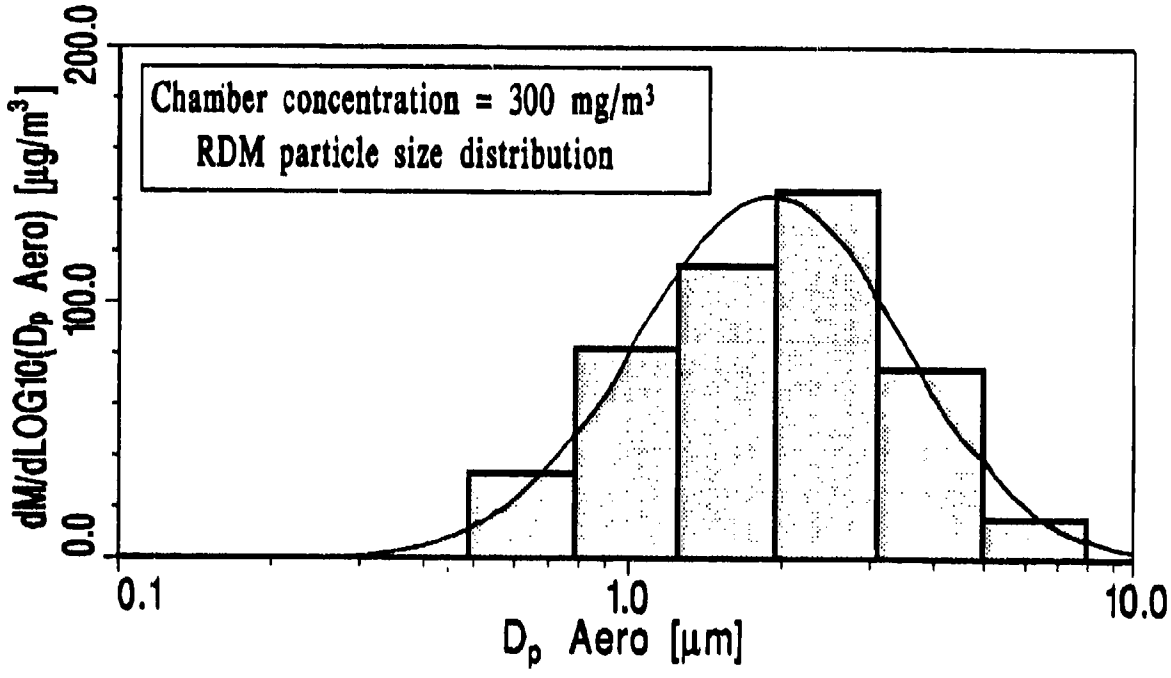


Figure 10. ITP Cascade Impactor Curve (0.49-8.0 µm).

TABLE 9. DISTFIT REPORT FOR ITP CASCADE IMPACTOR CURVE (0.49 to 8.0 µm)

Moment type (x-axis):	D_p Aero (µm)
Included intervals:	0.49 – 8.0 (µm) (1 – 6)
Weighting type (y-axis):	M (µg/m³)
Converted data type:	Interval

Geometric Measures of Central Tendency

	Total	Mean	Median	Mode	Std Dev
Discrete data	267.0	3.6	4.12	0.51	1.77
Analytical data	50.3	1.21	1.3	–	1.46
Function	93.3	1.88	1.89	–	1.82

Analytical Fitting Functions

Type	M	MMD	SD_g	ChiSq
1: LNDF	93.6	1.89	1.83	0.591

Fractional Classifications

Respirable M Fraction	Discrete Data	Analytical Data	Function
ACGIH based on LNDF:	0.445	0.982	0.804
ACGIH based on polynomial:	0.446	0.9	0.754

Size Classification	Discrete Data	Analytical Data	Function
Fraction <1 (µm):	0.03394	0.147	0.147
Fraction >1 (µm):	0.966	0.853	0.853

Discrete Data

D_p Aero	Converted Data	Analytical Data	Percent Difference	Correction
0.49	4.07	5.802	-42.55	1.0
0.79	10.9	16.17	-48.37	1.0
1.25	27.19	27.17	0.084985	1.0
2.0	50.67	24.92	50.81	1.0
3.17	79.42	13.4	83.12	1.0
5.03	94.27	4.131	95.62	1.0
8.0				

All three cascade impactor curves were superimposed in Figure 11, indicating their relative distributions. Finally, all three curves were merged and plotted as a single curve in Figure 12. This merged curve indicates MMADs of 0.203, 2.10, and 20.3 µm with corresponding σ_gs of 1.44, 1.82, and 1.10 µm (Table 10).

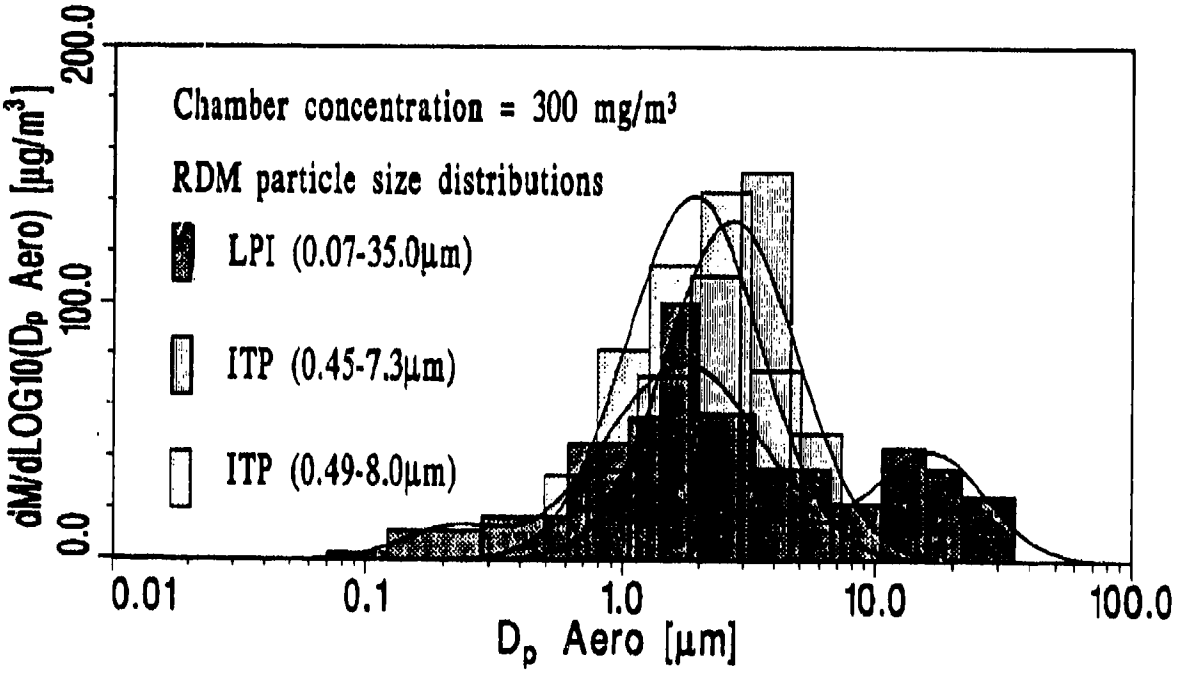


Figure 11. ITP and LPI Cascade Impactor Curves.

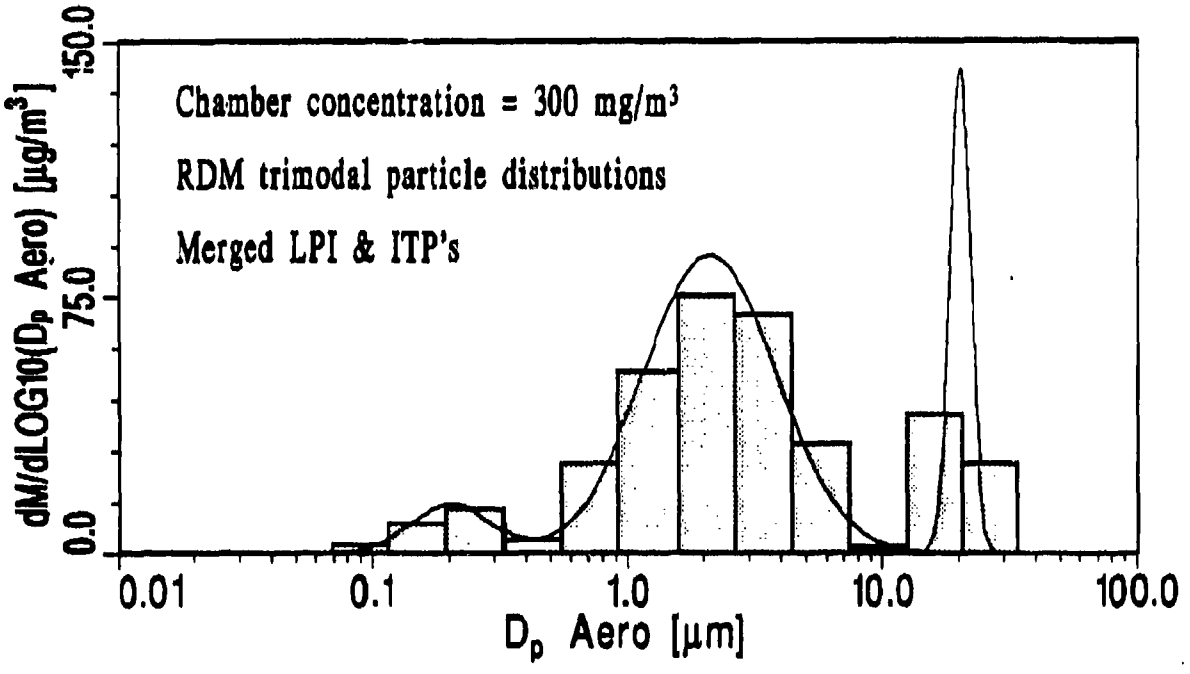


Figure 12. Merged Cascade Impactor Curves.

TABLE 10. DISTFIT REPORT FOR MERGED CASCADE IMPACTOR CURVES

Moment type (x-axis): D_p Aero (μm)
Included intervals: 0.070 – 35.0 (μm) (1 – 12)
Weighting type (y-axis): M (μg/m³)
Converted data type: Interval

Geometric Measures of Central Tendency

	Total	Mean	Median	Mode	Std Dev
Discrete data	80.1	2.73	2.54	4.67	3.55
Analytical data	77.6	2.74	2.37	–	3.55
Function	76.5	2.68	2.37	–	3.54

Analytical Fitting Functions

Type	M	MMD	SD_g	ChiSq
1: LNDF	5.75	0.203	1.44	0.0309
2: LNDF	56.8	2.1	1.82	0.0309
3: LNDF	15.1	20.3	1.1	0.0309

Fractional Classifications

Respirable M Fraction	Discrete Data	Analytical Data	Function
ACGIH based on LNDF:	0.61	0.631	0.64
ACGIH based on polynomial:	0.574	0.592	0.599

Size Classification	Discrete Data	Analytical Data	Function
Fraction < 1 (μm):	0.171	0.153	0.153
Fraction > 1 (μm):	0.829	0.847	0.847

Discrete Data

D_p Aero	Converted Data	Analytical Data	Percent Difference	Correction
0.070	0.5319	0.3793	28.7	1.0
0.1175	2.016	2.311	-14.61	1.0
0.1972	3.001	2.587	13.79	1.0
0.331	0.9501	1.185	-24.75	1.0
0.5556	5.928	4.226	28.71	1.0
0.9325	12.04	12.75	-5.899	1.0
1.565	17.01	19.02	-11.84	1.0
2.627	15.77	14.0	11.21	1.0
4.41	7.273	5.076	30.21	1.0
7.402	0.3987	0.904	-126.8	1.0
12.42	9.184	9.183	0.011806	1.0
20.85	5.955	5.956	-0.017833	1.0

SECTION 4

CONCLUSIONS

The high σ_g seen for all particle size distributions resulted from the two dyes in each mixture fractionating at different ECDs. The particle size distributions for the RDM are trimodal and describing them with a single MMAD and σ_g is why the deviations are large.

The chamber distributions had high variabilities because of the poor mixing characteristics of the Hazleton 2000 chamber with larger particle sizes (greater than 2.0 μm). The chamber mixing can be improved by increasing either the chamber air changes (which was not practical with these high concentrations) or the chamber turbulence (by adding an additional blower inside the chamber). The latter solution was not discovered until after the studies were already underway. These chamber modifications would have changed the exposure parameters in the middle of the 90-day study. So, to maintain consistency throughout the studies, the animal rotational frequency was increased to once per week.

SECTION 5

ACKNOWLEDGMENTS

Major contributions were provided by Joseph Sandy, Dock Terrell, Bob Jones, and Leon Walsh III of the Inhalation Exposure group of Mantech Environmental Technology, Inc. (METI). Technical editing and graphics assistance was provided by the Technical Publications staff of METI.

SECTION 6

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SECTION 7

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